

Microbiome change by symbiotic invasion in lichens

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Biological soil crusts (BSC) seal the soil surface from erosive forces in many habitats where plants cannot compete. Lichens symbioses of fungi and algae often form significant fraction of these microbial assemblages. In addition to the fungal symbiont, many species of other fungi can inhabit the lichenic structures and interact with their hosts in different ways, ranging from commensalism to parasitism. More than 1800 species of lichenicolous (lichen-inhabiting) fungi are known to science. One example is *Diploschistes muscorum*, a common species in lichen-dominated BSC that infects lichens of the genus *Cladonia*. *D. muscorum* starts as a lichenicolous fungus, invading the lichen *Cladonia symphylicarpa* and gradually develops an independent *Diploschistes* lichen thallus. Furthermore, bacterial groups, such as Alphaproteobacteria and Acidobacteria, have been consistently recovered from lichen thalli and evidence is rapidly accumulating that these microbes may generally play integral roles in the lichen symbiosis.

Here we describe lichen microbiome dynamics as the parasitic lichen *D. muscorum* takes over *C. symphylicarpa*. We used high-throughput 16S rRNA gene and photobiont-specific ITS rDNA sequencing to track bacterial and algal transitions during the infection process, and employed fluorescence *in situ* hybridization to localize bacteria in the *Cladonia* and *Diploschistes* lichen thalli. We sampled four transitional stages, at sites in Sweden and Germany: A) *Cladonia* with no visible infection, B) early infection stage defined by the first visible *Diploschistes* thallus, C) late-stage infection with parts of the *Cladonia* thallus still identifiable, and D) final stage with a fully developed *Diploschistes* thallus,

A gradual microbiome shift occurred during the transition, but fractions of *Cladonia*-associated bacteria were retained during the process of symbiotic reorganization. Consistent changes observed across sites included a notable decrease in the relative abundance of Alphaproteobacteria with a concomitant increase in Betaproteobacteria. Armatimonadia, Spartobacteria and Acidobacteria also decreased during the infection of *Cladonia* by *Diploschistes*. The lichens differed in photobiont specificity. *C. symphylicarpa* was associated with the same algal species at all sites, but *D. muscorum* had a flexible strategy with different photobiont combinations at each site. This symbiotic invasion system suggests that partners can be reorganized in BSC and selected for maintaining potential roles rather than depending on particular species.